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Case Report—

Parvovirus-Associated Cerebellar Hypoplasia and Hydrocephalus in Day Old Broiler Chickens

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SUMMARY. Cerebellar hypoplasia and hydrocephalus were identified in day old broiler chickens showing nervous signs, impaired mobility, and diarrhea. At postmortem examination, brains of chickens were misshapen and cerebellums were smaller than normal. Microscopically, cerebellar folia were reduced in size and irregularly shaped, and the ventricles were widely distended. Affected cerebellums had focal areas along the base of folia where the internal granular cell layer had been lost, and Purkinje cells were disorganized and located within the molecular layer. Parvovirus DNA was detected by polymerase chain reaction in three of nine brains with oligonucleotide primers designed for amplification of chicken and turkey parvoviruses. On the basis of phylogenetic analyses, the detected virus was most closely related to chicken parvoviruses. These findings suggest that a chicken parvovirus might cause a neurologic disease of young chickens characterized by cerebellar hypoplasia and hydrocephalus; however, its role as the cause of the disease remains to be confirmed.

RESUMEN. *Reporte de Caso*—Hipoplasia cereberal e hidrocefalia asociadas con un parvovirus en pollos de engorde de un día de edad.

Se identificó hipoplasia cerebelar e hidrocefalia en pollos de engorde de un día de edad que mostraban signos nerviosos, dificultad de movimiento y diarrea. Al examen postmortem, los cerebros de las aves estaban deformes y los cerebelos estaban más pequeños de lo normal. Microscópicamente, la folia cereberal estaba reducida en tamaño y de forma irregular, mientras que los ventrículos estaban ampliamente distendidos. Los cerebelos afectados presentaron focos a lo largo de la base de la folia donde la capa granular interna se había perdido y las células de Purkinge estaban desorganizadas y se localizaban dentro de la capa molecular. Mediante la prueba de reacción en cadena de la polimerasa, se detectó ADN de un parvovirus en tres de nueve cerebros utilizando iniciadores diseñados para amplificar parvovirus de pollo y de pavo. Con base en el análisis filogenético, los virus detectados estaban más cercanamente relacionados a los parvovirus de pollos. Estos hallazgos sugieren que los parvovirus de pollo pueden causar enfermedad neurológica en pollos jóvenes, caracterizada por hipoplasia cereberal e hidrocefalia, sin embargo, su papel como la causa de la enfermedad debe confirmarse.

Key words: parvovirus, broiler chickens, cerebellar hypoplasia, hydrocephalus

Abbreviations: AV = Aino virus; CA = cerebellar abiotrophy; CH = cerebellar hypoplasia; CNS = central nervous system; FFPE = formalin-fixed, paraffin-embedded; FGV = fowl glioma–inducing virus; NS = nonstructural

Neurologic disease is a common cause of early culling and death in both chickens and turkeys. Established etiologies include avian encephalomyelitis (picornavirus), bacterial encephalitis (e.g., *Salmonella* sp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* sp.), nutritional encephalomalacia (vitamin E deficiency), encephalocele/exencephaly, and pox vaccination reaction (1,7,20,21). These diseases can usually be differentiated by gross and microscopic lesions in the brain. Hypoglycemia and poult flip-over syndrome result in neurologic signs with less well understood etiologies and absence of lesions in the central nervous system (CNS).

Two congenital neurologic diseases characterized by grossly small cerebellums are cerebellar hypoplasia (CH) and cerebellar abiotrophy (CA). Although these are common in mammalian neonates (27), reports in birds are rare. Cerebellar hypoplasia has been experimentally induced by yolk sac inoculation of chicks with both fowl glioma–inducing virus (FGV), a member of avian leukosis virus subgroup A (31), and Aino virus (AV), a virus of the family *Bunyaviridae* known to cause fetal deformities, CH, and hydranencephaly in cattle and sheep (19). Naturally occurring cerebellar hypoplasia in chickens or turkeys has not previously been reported.

Parvovirus is known to cause cerebellar hypoplasia in both kittens and puppies, although in the dog, the enteric form of the virus is most prevalent (24,26,27). Parvovirus is also a well-characterized enteric pathogen of young geese and Muscovy ducks (6,14) and has been described in both turkeys and chickens (12,13,15,16,17,18). Parvovirus has been associated with poult enteritis complex in turkeys and runting-stunting syndrome in chickens (32,33). The partial genome sequences of both the turkey and chicken enteric parvoviruses have recently been described and are closely related to each other, representing novel members of the *Parvoviridae* subfamily (33). Zsak *et al.* (32) have also characterized the ubiquitous nature of enteric parvovirus in commercial flocks in the United States. The virus is transmitted both vertically and horizontally and can become systemic (15); however, detection of the virus in neurologic tissues has not been previously reported.

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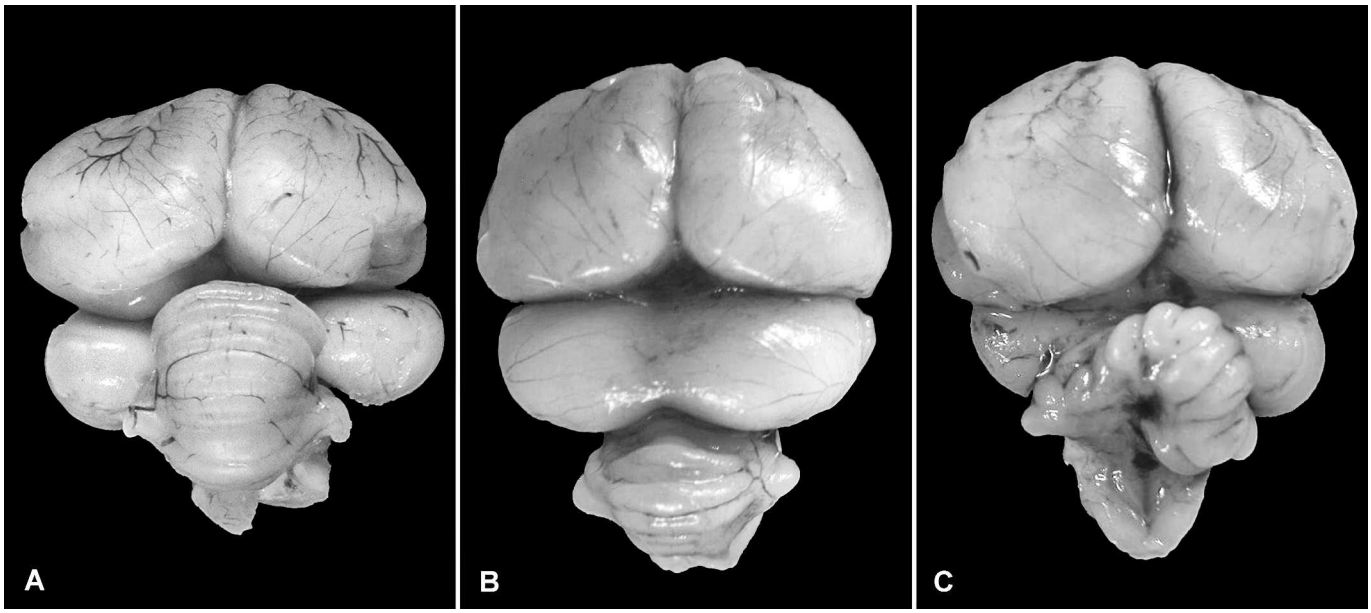

Fig. 1. Brains from day old chicks. (A) Normal; (B) small cerebellum and dorsally fused optic lobes; (C) malformed cerebellum.

This paper describes the first case of CH and hydrocephalus in commercial broiler chickens showing both enteric disease and neurologic signs. Evidence for a possible etiologic association of chicken parvovirus in this disease was provided by detection of the virus in affected brains by the PCR procedure.

CASE REPORT

Case history. Fourteen 1-day-old broiler chickens were presented for diagnostic evaluation to the Rollins Animal Disease Diagnostic Laboratory in Raleigh, North Carolina. Eleven chicks were alive and three were dead. Chickens were weak and abnormally small, had pasty vents, and mobility was impaired (''bad legs''). Eight of the 11 live birds were lying on their sides, with paddling of the legs. The birds were also experiencing increased mortality and diarrhea (enteric disease). Chickens were from one of four houses of a North Carolina farm, each house holding approximately 22,500 birds; no problems were seen in the other three houses. The percent morbidity in the house was not assessed; however, the grower noted morbidity was most prevalent during the first 3 days and declined after this time. The farm average weekly mortality was 1.23% (week 1), 0.4% (week 2), 0.39% (week 3), 0.25% (week 4), and 0.14% (week 5), consistent with the grower's observations.

Gross pathology. Five chickens had abnormal brains; both optic lobes were swollen and fused on the dorsal surface of the brain. In general, cerebellums were smaller than normal, and in one case, the cerebellum was grossly disfigured (Fig. 1). In seven chickens, the koilin layer of the gizzard was bile stained and had areas of hemorrhage. No other lesions were seen.

Histopathology. Histologic sections were prepared from the brains of nine live chicks. Of these, eight birds were on their sides and paddling. Consistent with the grossly small cerebellums, the folia were shortened and misshapen (Fig. 2A,B). Purkinje cells were usually organized in a single cell layer at the interface of the molecular and internal granular layers. However, in the most affected brains, there was focal loss of the internal granular cell layer along the base of some folia (Fig. 2B,C). In these areas, Purkinje cells were disorganized and located within the molecular layer. No active inflammation was associated with the lesions. In four of nine affected brains, the ventricles were dilated, indicating hydrocephalus (Fig. 2D). Lesions were also found in the ventriculus and included marked cavitation and focal hemorrhage in the koilin layer, but these were considered nonspecific because they are commonly seen in birds that are off feed.

PCR procedure. Formalin-fixed, paraffin-embedded (FFPE) sections of whole brain tissue from nine 1-day-old broiler chickens were used for DNA extraction. The procedure for whole brain extraction was performed with the QuickExtract™ FFPE DNA Extraction Kit (EpiCentre Biotechnologies, Madison, WI) according to the manufacturer's instructions.

A previously described PCR procedure that amplifies a 560-bp product within the nonstructural (NS) gene region of chicken and turkey parvoviruses was used (32). Positive control DNA consisted of an intestinal homogenate obtained from parvovirus-inoculated chickens (32). Negative controls included DNA extracted from brain sections of three normal chickens and a no-template control (nuclease-free water). The PCR procedure resulted in 560-bp products for three of nine brains. Direct nucleotide sequencing of the 560-bp products revealed that the nucleotide sequence corresponded to the NS gene region of chicken and turkey parvoviruses (GenBank GQ260159). Interestingly, the brain that appeared most normal histologically was most strongly positive for parvovirus by PCR, which might reflect the mild and temporal nature of the infection and difficulty identifying parvovirus postinfection by PCR.

Phylogenetic analysis. PCR amplicons were used for phylogenetic analysis, which was done according to described methods (32). Phylogenetic analysis demonstrated that the parvovirus amplicon in CH brains (ChbrNC) was closely related to chicken parvoviruses (Fig. 3).

DISCUSSION

In this study, parvovirus was detected by PCR analysis in day old commercial broiler chickens with CH and hydrocephalus. Fourteen commercial broiler chickens were submitted for postmortem

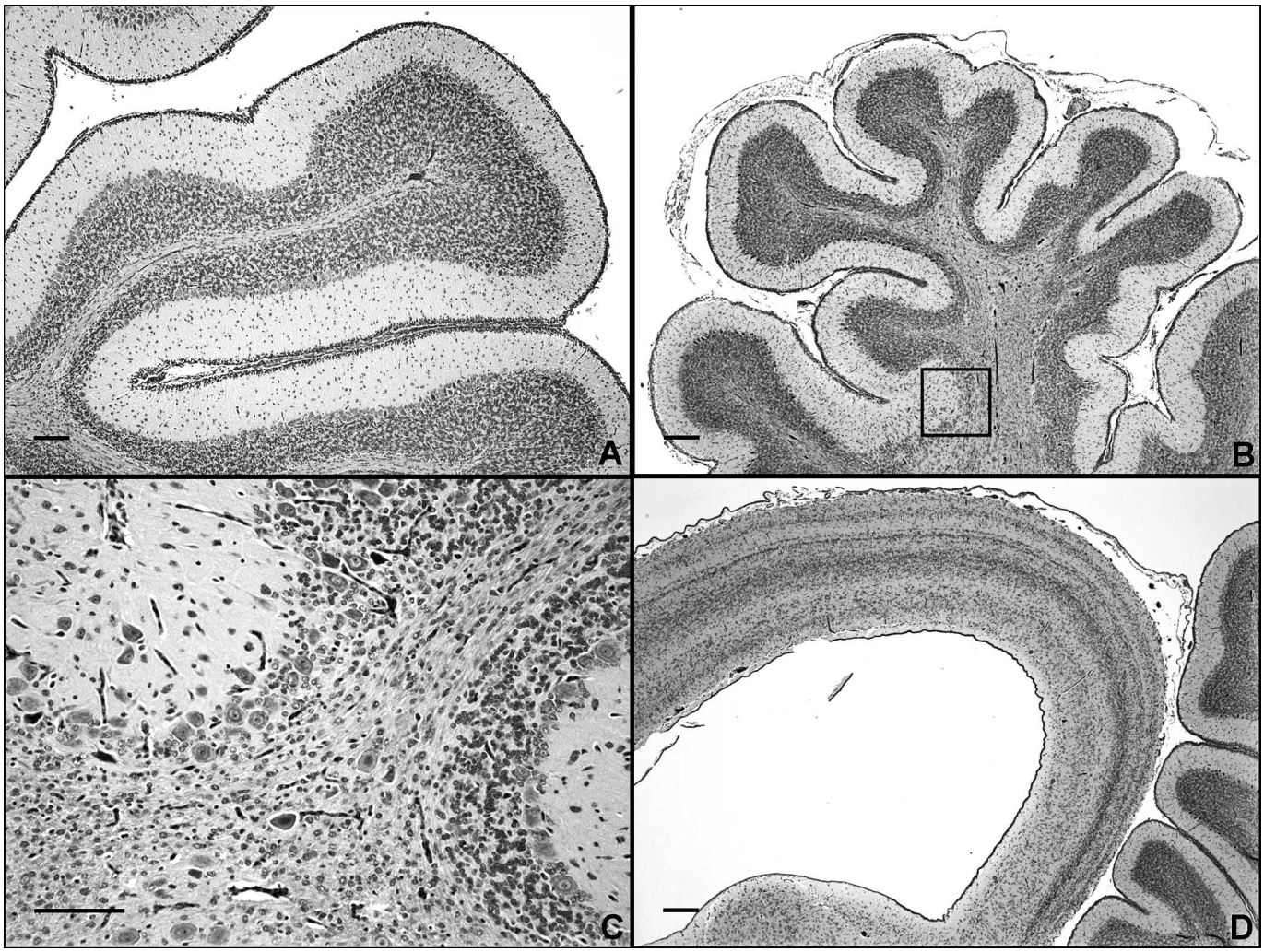

Fig. 2. Cerebellums from young chicks. (A) Normal folium of a 3-day-old chick. (B) Affected cerebellum, 1-day old chick. Folia are shortened and misshapen (compare with panel A taken at same magnification). Focal loss of the internal granular layer (box). (C) Higher magnification of area in box of panel B. Base of affected folium showing the lack of internal granular layer cells and displacement of Purkinje cells into the molecular layer. (D) Affected cerebellum, 1-day old chick. Marked dilatation of the third ventricle indicative of hydrocephalus. Bars in A, B, and D = 250 μm; bar in C = 150 μm.

examination to investigate runting-stunting, pasty vents, and impaired mobility. At postmortem examination, eight of 11 live birds showed neurologic signs (lying on their sides and paddling), prompting examination of the brains. Brains of nine birds (eight lying on their sides and paddling and one remaining upright) were examined. Gross brain lesions in five of the chicks were immediately obvious and included small or deformed cerebellums and swollen, dorsally fused optic lobes.

Two different early pathologic processes can result in small cerebellums: cerebellar hypoplasia and cerebellar abiotrophy. These can be initially distinguished by following the temporal pattern of clinical signs. Clinical signs are progressive with CA, and although CH can also be progressive (2,25), congenital CH is generally static, with signs remaining constant or diminishing as the animal adjusts. The grower's description of ''bad legs'' might have been the first neurologic sign seen in these chicks, which advanced to ataxia and paddling only under the stressful conditions of transportation. Because the grower reported that these clinical signs were most prevalent only during the first 3 days and had completely ceased after 1–2 wk, this further suggests congenital CH to be the most likely pathologic process.

Although a genetic anomaly could be responsible for the lesions seen, congenital CH is most often caused by viruses. This condition is well described in mammals and shows histopathologic lesions primarily in the cortex of the cerebellar folia, which vary with viral etiology. For example, bovine viral diarrhea virus, a pestivirus that causes CH in calves, is characterized by a nonsuppurative inflammation of the cerebellar cortex and adjacent leptomeninges, widespread necrosis of neuronal elements and neuropil, and hemorrhages and severe edema of the cerebellar folia and medullary white matter (23). Hog cholera, also a pestivirus, causes hypomyelinogenesis along with CH in pigs (3). In both dogs and cats, parvovirus targets rapidly dividing granular cells, causing necrosis of the external granular layer (24,26,27). The resulting hypoplastic molecular and granular cell layers cause CH. In cats, but not dogs, Purkinje cells also become infected by the virus via a different cytopathology and become haphazardly arranged adjacent to the white matter of the folia. Purkinje cell arrangement is normal in dogs.

The small cerebellums in these day old chickens are most consistent with virus-induced CH. Two viruses, Aino virus and fowl glioma–inducing virus have been shown to cause CH in

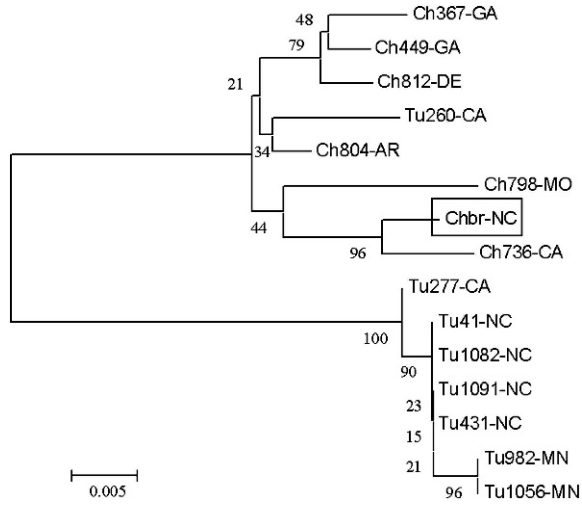

Fig. 3. Phylogenetic tree showing the relationship of the parvovirus DNA identified in affected day old chick brains (ChbrNC) to other chicken and turkey parvoviruses identified from intestinal samples on U.S. farms. The evolutionary history was inferred by the neighbor-joining method (22). The optimal tree with the sum of branch length = 0.12314265 is shown. Percentages of replicate trees, in which associated taxa clustered together in the bootstrap test (1000 replicates), are shown next to the branches (5). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by the maximum composite likelihood method (29) and are in the units of the number of base substitutions per site. There were 561 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4 (28).

experimentally infected chickens. Gross and histopathologic brain lesions in chick embryos inoculated *in ovo* with AV are severe and result in early embryonic skeletal and brain abnormalities (19). In the cerebellum, differentiating cortical neurons become disorganized due to severe parenchymal liquefactive necrosis, thinning of the cerebellar parenchyma because of dilatation of the fourth ventricle and temporal gliosis; these lesions were not observed in the chickens in this study. In contrast, FGV infection results in cerebellar lesions that are similar to those seen with mammalian parvovirus, including a diffuse absence of internal granular cells and thinning of this layer. Disorganization of the Purkinje cell layer is also seen, but it is the result of decreased numbers and irregular arrangement of Bergmann's fibers rather than direct virus-induced cytopathology (31). Fowl gliomas were observed in the cerebra of all experimentally infected birds and in the cerebellum of one bird; these were not detected in chickens evaluated in this study. Neither AV nor FGV has been identified as a cause of naturally occurring CH in commercial flocks.

Chickens in this study had cerebellums that were, in general, smaller than normal, with folia that were shortened and irregular. The most distinctive cortical cerebellar feature was disruption of the architecture at the base of some folia, characterized by focal areas of missing internal granular layers and disorganization of Purkinje cells. Inflammation was not a feature of the lesions in the brain. Lesions in the cerebellums of these chicks were similar to both mammalian parvovirus–induced lesions and those that developed in chicks following *in ovo* infection with FGV.

Marked dilation of the third ventricle (hydrocephalus) also occurred in the day old chicks. Hydrocephalus can be internal, in which obstruction of CSF flow within the ventricular system causes ventricular enlargement and brain atrophy, or communicating, in which decreased resorption of CSF at the arachnoid villi causes expansion of the ventricular system and subarachnoid space. Communicating hydrocephalus caused by vitamin A deficiency has been reported in an adult Goffin's cockatoo (10), and internal hydrocephalus has been reported in Polish fowl because of abnormal brain growth changes (8). Viral infections during pregnancy are known to alter normal CNS development in offspring (9), and fetal parvovirus infection has been associated with hydrocephalus in both human beings (11) and cats (26,30). Aqueductal stenosis, a common malformation associated with the fusion of the two rostral colliculi (optic lobes in birds) that can follow prenatal inflammatory processes (27), is a likely cause for the dilated third ventricle seen in these chicks.

Both avian parvovirus (32,33) and leukosis virus serotype A (4) are common in the field and can be transmitted both vertically and horizontally. The very young age of the affected birds, the brain lesions, and the fact that runting-stunting syndrome, characterized by slow growth, diarrhea, and mortality was a major concern in the flock under consideration, prompted us to analyze the affected brains for parvoviral DNA. Parvovirus was detected in three of the nine brain samples. The inability to demonstrate parvovirus DNA in the other brain sections might have resulted from small amounts of virus in the tissue or possible DNA degradation from formalin fixation. Because the amount of tissue available for DNA extraction from day old cerebellum is very small, whole brain slices were used in the extraction process. This precluded localization of the virus to just the cerebellum and obtaining information about the etiology of the hydrocephalus.

Phylogenetic analysis indicated that the detected parvoviruses were similar to chicken parvoviruses previously identified in intestinal contents collected in the United States. This identification, along with neurologic signs and enteric disease at the time of diagnostic investigation, makes parvovirus a likely agent as the direct cause of the CH and hydrocephalus. However, FGV cannot be ruled out at this time. Immunohistochemical analysis for parvovirus localization and disease reproduction following Koch's postulates are needed to definitively establish parvovirus as the etiologic agent. Although parvoviruses appear to be common in chickens and turkeys, on the basis of previous findings, it seems strange that CH has not previously been described. However, infrequent evaluation or failure to closely evaluate the gross and microscopic appearance of brains of young chickens could explain the failure to recognize this condition in the past. Also, parvoviral infection with subsequent vertical transmission during lay might be rare because of the frequent infection of young chickens with the virus. Of particular interest would be to evaluate the genetics of the parvoviruses identified in the present case more fully to determine whether these viruses might represent unique genotypes associated with neurologic infection.

The congenital nature of this disease suggests that the etiologic agent was vertically transmitted; transovarial transmission of chicken parvovirus has been reported (17). Parvoviruses originating in the gut can become viremic and settle in multiple organs (6,14), however, and horizontal transmission from hatchery contamination during *in ovo* vaccination should be considered.

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